

WHAT IS CLAIMED IS:

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B1 5 1. A method for evaluating the binding of a probe to a target molecule, said method comprising comparing the amount of binding of molecules in a first sample to the probe with the amount of binding of molecules in a second sample to the probe, wherein:

- (a) the first sample comprises a plurality of molecules of the same target molecule; and
- (b) the second sample comprises a plurality of different target molecules.

10 2. The method of claim 1 wherein the first sample is a substantially pure sample of the molecules of the same target. B

3. The method of claim 2 wherein the first sample is at least 75% pure.

15 4. The method of claim 2 wherein the first sample is at least 90% pure.

5. The method of claim 2 wherein the first sample is at least 95% pure.

6. The method of claim 2 wherein the first sample is at least 99% pure.

Sub
B2 20 7. The method of claim 1 wherein each different target molecule in the second sample is different from the same target molecules in the first sample.

8. The method of claim 1 wherein the sensitivity of the probe is determined.

25 9. The method of claim 8 wherein the sensitivity of the probe is determined from the amount of binding of molecules of the particular target in the first sample to the probe.

30 10. The method of claim 1 wherein the specificity of the probe is determined.

35 11. The method of claim 10 wherein the specificity of the probe is determined from the ratio of the amount of binding of the same target molecules in the first sample to the probe to the amount of binding of molecules of the different target molecules in the second sample to the probe.

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12. The method of claim 1 wherein the molecules of the same target molecule in the first sample are detectably labeled.

13. The method of claim 1 wherein the molecules of the different target
5 molecules in the second sample are detectably labeled.

14. The method of claim 12 or 13 wherein the molecules are detectably labeled with a fluorescent molecule.

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10 15. The method of claim 1 wherein:
(a) the molecules of the same target molecule in the first sample are detectably labeled with a first label, and
(b) the molecules of the different target molecules in the second sample are detectably labeled with a second label,
15 the first label being distinguishable from the second label.

16. The method of claim 15 wherein:
the first label is a first fluorescent molecule, and
the second label is a second fluorescent molecule.

20 17. The method of claim 1 wherein the probe is attached to a surface of a support.

18. The method of claim 1 wherein the probe is one of a plurality of probes.

25 sub A12 19. The method of claim 18 wherein the plurality of probes comprises an array of probes,
said having a support with at least one surface, and
wherein each probe is attached to the surface of the support in a different location on
30 said surface.

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20. The method of claim 1 wherein the probe is a polynucleotide probe having a particular nucleotide sequence.

35 21. The method of claim 20 wherein the molecules of the same target molecule in the first sample are polynucleotide molecules.

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22. The method of claim 21 wherein the particular nucleotide sequence of the polynucleotide probe is complementary to at least a portion of the nucleotide sequence of the polynucleotide molecules in the first sample.

5 23. The method of claim 21 wherein the molecules of the different target molecules in the second sample are polynucleotide molecules having a polynucleotide sequence that is different from the nucleotide sequence of the polynucleotide molecules in the first sample.

10 24. The method of claim 20 wherein the polynucleotide probe is attached to a surface of a support.

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25. The method of claim 20 wherein the polynucleotide probe is one of a plurality of polynucleotide probes having different nucleotide sequences.

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26. The method of claim 25 wherein the plurality of polynucleotide probes comprises an array of polynucleotide probes, said having a support with at least one surface, and wherein each polynucleotide probe is attached to the surface of the support in a
20 different location on said surface.

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27. A method for evaluating the binding of a polynucleotide probe having a particular nucleotide sequence to a target polynucleotide, said method comprising comparing the amount of hybridization of polynucleotides in a first sample to the
25 polynucleotide probe with the amount of hybridization of polynucleotides in a second sample to the polynucleotide probe, wherein:

- 30 (a) the first sample comprises a plurality of the same target polynucleotide having a target nucleotide sequence; and
(b) the second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule has a different nucleotide sequence.

28. The method of claim 27 wherein the particular nucleotide sequence of the polynucleotide probe is complementary to at least a portion of the target nucleotide
35 sequence of the target polynucleotide in the first sample.

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29. The method of claim 27 wherein the target polynucleotide in the first sample corresponds to a gene or gene transcript of a cell or organism, or to mRNA, cDNA or cRNA derived therefrom.

5 30. The method of claim 27 wherein the plurality of different polynucleotide molecules in the second sample corresponds to a plurality of different genes or gene transcripts of a cell or organism.

10 31. The method of claim 27 wherein the first sample is a substantially pure sample of molecules of the target polynucleotide.

32. The method of claim 31 wherein the first sample is at least 75% pure.

33. The method of claim 31 wherein the first sample is at least 90% pure.

34. The method of claim 31 wherein the first sample is at least 95% pure.

35. The method of claim 31 wherein the first sample is at least 99% pure.

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36. The method of claim 31 wherein each different polynucleotide molecule is the second sample has a nucleotide sequence different from the target nucleotide sequence.

37. The method of claim 36 wherein:

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(a) the target polynucleotide in the first sample corresponds to a gene or gene transcript of a cell or organism; and
(b) the second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism,

wherein the deletion mutant of the cell or organism does not express the gene or gene transcript corresponding to the target polynucleotide in the first sample.

30 38. The method of claim 31 wherein the plurality of different polynucleotide molecules in the second sample comprises:

- 35 (a) polynucleotide molecules having a nucleotide sequence that is the same as the target nucleotide sequence in the first sample, and
(b) a plurality of different polynucleotide molecules each having a different nucleotide sequence that is different from the target nucleotide sequence.

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39. The method of claim 38 wherein:
- (a) the target polynucleotide corresponds to a gene or gene transcript of a cell or organism; and
 - (b) the second sample comprises a polynucleotide sample from a wild-type strain of the cell or organism,
- wherein the wild-type strain of the cell or organism expresses the gene or gene transcript corresponding to the target polynucleotide.

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40. The method of claim 27 wherein:
- (a) the first sample further comprises polynucleotide molecules having a nucleotide sequence different from the target nucleotide sequence of said same target polynucleotide; and
 - (b) the second sample lacks said same target polynucleotide.

41. The method of claim 40 wherein each different polynucleotide molecule in the second sample has a nucleotide sequence different from the target nucleotide sequence.

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42. The method of claim 41 wherein:
- (a) the target polynucleotide corresponds to a gene or gene transcript of a cell or organism;
 - (b) the first sample comprises a polynucleotide sample from a wild-type strain of the cell or organism which expresses the gene or gene transcript corresponding to the target polynucleotide; and
 - (c) the second sample comprises a polynucleotide sample from a deletion mutant of the cell or organism which does not express the gene or gene transcript corresponding to the target polynucleotide.

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43. The method of claim 27 wherein:
- (a) the first sample further comprises polynucleotide molecules having a nucleotide sequence different from the target nucleotide sequence of said same target polynucleotide; and
 - (b) the second sample comprises:

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- (i) polynucleotide molecules having a nucleotide sequence that is the same as the target nucleotide sequence, and
- (ii) a plurality of different polynucleotide molecules, each different polynucleotide molecule having a different nucleotide sequence that is different from the target nucleotide sequence,

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wherein the amount of polynucleotide molecules in the first sample having the target nucleotide sequence differs by at least a factor of two from the amount of polynucleotide molecules in the second sample having the target nucleotide sequence.

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44. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample having the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample having the target nucleotide sequence by at least a factor of four.

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45. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample having the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample having the target nucleotide sequence by at least a factor of eight.

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46. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample having the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample having the target nucleotide sequence by at least a factor of twenty.

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47. The method of claim 43 wherein the amount of polynucleotide molecules in the first sample having the target nucleotide sequence differs from the amount of polynucleotide molecules in the second sample having the target nucleotide sequence by at least a factor of 100.

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Sub Cw 48. The method of claim 43 wherein the amount of each different polynucleotide molecule in the plurality of different molecules of the first sample differs from the amount of the corresponding different polynucleotide molecule in the plurality of different polynucleotide molecules of the second sample by no more than a factor of 100.

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49. The method of claim 43 wherein the amount of each different polynucleotide molecule in the plurality of different molecules of the first sample differs from the amount of the corresponding different polynucleotide molecule in the plurality of different polynucleotide molecules of the second sample by no more than a factor of 10.

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50. The method of claim 43 wherein the amount of each different polynucleotide molecule in the plurality of different molecules of the first sample differs from the amount of the corresponding different polynucleotide molecule in the plurality of different polynucleotide molecules of the second sample by no more than 50%.

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51. The method of claim 43 wherein the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the first sample differs from the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the second sample by no more than a factor of two.

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52. The method of claim 43 wherein the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the first sample differs from the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the second sample by no more than 50%.

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53. The method of claim 43 wherein the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the first sample differs from the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the second sample by no more than 10%.

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54. The method of claim 43 wherein the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the first sample differs from the mean abundance of the different polynucleotide molecules in the plurality of different polynucleotide molecules of the second sample by no more than 1%.

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55. The method of claim 27 wherein the sensitivity of the polynucleotide probe is determined.

56. The method of claim 55 wherein the sensitivity of the polynucleotide probe is determined from the amount of hybridization of target polynucleotide molecules in the first sample to the polynucleotide probe.

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57. The method of claim 27 wherein the specificity of the polynucleotide probe is determined.

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58. The method of claim 57 wherein the specificity of the polynucleotide probe is determined from the ratio of the amount of hybridization of target polynucleotide molecules in the first sample to the polynucleotide probe to the amount of hybridization of polynucleotide molecules in the second sample to the polynucleotide probe.

59. The method of claim 27 wherein the target polynucleotide molecules in the first sample are detectably labeled.

60. The method of claim 27 wherein the polynucleotide molecules in the second sample are detectably labeled.

61. The method of claim 59 or 60 wherein the polynucleotide molecules are labeled with a fluorescent molecule.

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62. The method of claim 27 wherein:

(a) the target polynucleotide molecules in the first sample are labeled with a first label; and

(b) the polynucleotide molecules in the second sample are labeled with a second label,

the first label being distinguishable from the second label.

63. The method of claim 62 wherein:
the first label is a first fluorescent molecule, and
the second label is a second fluorescent molecule.

64. The method of claim 27 wherein the polynucleotide probe is attached to a surface of a support.

65. The method of claim 27 wherein the polynucleotide probe is one of a plurality of polynucleotide probes.

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66. The method of claim 65 wherein the plurality of polynucleotide probes comprises an array of polynucleotide probes,
said array having a support with at least one surface, and
wherein each probe is attached to the surface of the support in a different location on said surface.

67. A method for evaluating the binding of a plurality of polynucleotide probes to a target polynucleotide wherein each polynucleotide probe in the plurality of polynucleotide probes has a particular nucleotide sequence,

said method comprising comparing the amount of hybridization of polynucleotides in a first sample to each polynucleotide probe in the plurality of polynucleotide probes with the amount of hybridization of polynucleotides in a second sample to each polynucleotide probe in the plurality of polynucleotide probes, wherein:

- (a) the first sample comprises a plurality of the same target polynucleotide having a target nucleotide sequence; and
- (b) the second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule has a different nucleotide sequence.

68. The method of claim 67 wherein the particular nucleotide sequence of each polynucleotide probe is complementary to at least a portion of the target nucleotide sequence of the target polynucleotide in the first sample.

69. The method of claim 67 wherein the sensitivity of each polynucleotide probe in the plurality of different polynucleotide probes is determined.

70. The method of claim 69 wherein the sensitivity of each polynucleotide probe in the plurality of polynucleotide probes is determined from the amount of hybridization of the same target polynucleotide molecules in the first sample to each polynucleotide probe in the plurality of polynucleotide probes.

71. The method of claim 69 wherein the specificity of each polynucleotide probe in the plurality of different polynucleotide probes is determined.

72. The method of claim 71 wherein the specificity of each polynucleotide probe in the plurality of polynucleotide probes is determined from the ratio of

- (a) the amount of hybridization of the same target polynucleotide molecules in the first sample to each polynucleotide probe to
- (b) the amount of hybridization of the plurality of different polynucleotide molecules in the second sample to each polynucleotide probe.

73. The method of claim 67 wherein each polynucleotide probe in the plurality of polynucleotide probes is attached to a surface of a support.

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74. The method of claim 67 wherein the plurality of polynucleotide probes comprises an array of probes,
said array having a support with at least one surface, and
wherein each probe in the plurality of probes is attached to the surface of the support
5 in a different location on said surface.

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75. The method of claim 67 wherein the first sample comprises two or more different target polynucleotide molecules
wherein none of the two or more different target polynucleotide molecules
10 hybridizes or cross-hybridizes to a probe that also hybridizes or cross-hybridizes to another one of the two or more different target polynucleotide molecules.

76. A method for evaluating hybridization conditions for one or more polynucleotide probes,
15 each of said one or more polynucleotide probes having a particular nucleotide sequence,
said method comprising comparing the amount of hybridization of polynucleotides in a first sample to each of the one or more polynucleotide probes with the amount of hybridization of polynucleotides in a second sample to each of the one or more
20 polynucleotide probes under particular hybridization conditions, wherein:
(a) the first sample comprises a plurality of the same target polynucleotide having a target nucleotide sequence; and
(b) the second sample comprises a plurality of different polynucleotide molecules wherein each different polynucleotide molecule has a different
25 nucleotide sequence.

77. The method of claim 76 wherein the sensitivity of each of the one or more polynucleotide probes under the particular hybridization conditions is determined.

30 78. The method of claim 77 wherein the sensitivity of each of the one or more polynucleotide probes is determined from the amount of hybridization of the plurality of the same target polynucleotide molecules in the first sample to each of the one or more polynucleotide probes under the particular hybridization conditions.

35 79. The method of claim 76 wherein the specificity of each of the one or more polynucleotide probes under the particular hybridization conditions is determined.

80. The method of claim 79 wherein the specificity of each of the one or more polynucleotide probes is determined from the ratio of:

- (a) the amount of hybridization of the plurality of the same target polynucleotide molecules in the first sample to each of the one or more polynucleotide probes under the particular hybridization conditions to
- (b) the amount of hybridization of the plurality of different polynucleotide molecules in the second sample to each of the one or more polynucleotide probes under the particular hybridization conditions.

81. The method of claim 1 further comprising, prior to said step of comparing, the steps of:

- (i) contacting the probe with the first sample under conditions conducive to binding;
- (ii) contacting the probe with the second sample under conditions conducive to binding;
- (iii) detecting any binding that occurs between the probe and molecules in the first sample; and
- (iv) detecting any binding that occurs between the probe and molecules in the second sample.

82. The method of claim 81, wherein said steps of contacting are performed concurrently.

83. The method of claim 82 wherein said steps of detecting are performed concurrently.

84. The method of claim 27 wherein:
polynucleotides in the first sample are labeled with a first label and polynucleotides in the second sample are labeled with a second label that is distinguishable from the first label;

and further comprising, prior to said step of comparing the steps of:

- (i) concurrently contacting the polynucleotide probe with the first sample and the second sample under conditions conducive to hybridization, and
- (ii) detecting any binding that occurs between the polynucleotide probe and polynucleotides in the first sample and the second sample.

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85. The method of any one of claims 81-84 wherein the second sample lacks said same target molecule or polynucleotide in said first sample.

5 86. A computer system for evaluating the binding of a probe to a target molecule,
said computer system comprising:
a memory; and
a processor element interconnected with the memory,
wherein the memory encodes one or more programs causing the processor element to
10 perform a method which comprises comparing the amount of binding of molecules in a first sample to the probe with the amount of binding of molecules in a second sample to the probe, and wherein:

- 15 (a) the first sample comprises a plurality of molecules of the same target molecule; and
(b) the second sample comprises a plurality of different target molecules.

20 87. A computer system for evaluating the binding of a polynucleotide probe, said computer system comprising:
a memory; and
a processor element interconnected with the memory,
wherein the memory encodes one or more programs causing the processor element to
perform a method which comprises comparing the amount of hybridization of
polynucleotides in a first sample to the polynucleotide probe with the amount of
hybridization of polynucleotides in a second sample to the polynucleotide probe, and
25 wherein:

- 30 (a) the first sample comprises a plurality of the same target polynucleotide having a target nucleotide sequence; and
(b) the second sample comprises a plurality of different polynucleotide molecules, each different polynucleotide molecules having a different nucleotide sequence.

88. A computer program product for use in conjunction with a computer having a memory and a processor element, the computer program product comprising a computer readable storage medium having a computer program mechanism encoded thereon, wherein
35 said computer program mechanism may be loaded into the memory and cause the processor element to execute a method which comprises comparing the amount of binding of

molecules in a first sample to the probe with the amount of binding of molecules in a second sample to the probe, and wherein:

- (a) the first sample comprises a plurality of molecules of the same target molecule; and
- 5 (b) the second sample comprises a plurality of different target molecules.

89. A computer program product for use in conjunction with a computer having a memory and a processor element, the computer program product comprising a computer readable storage medium having a computer program mechanism encoded thereon, wherein
10 said computer program mechanism may be loaded into the memory and cause the processor element to execute a method which comprises comparing the amount of hybridization of polynucleotides in a first sample to the polynucleotide probe with the amount of hybridization of polynucleotides in a second sample to the polynucleotide probe, and wherein:

- 15 (a) the first sample comprises a plurality of the same target polynucleotide having a target nucleotide sequence; and
- (b) the second sample comprises a plurality of different polynucleotide molecules, each different polynucleotide molecules having a different nucleotide sequence.

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